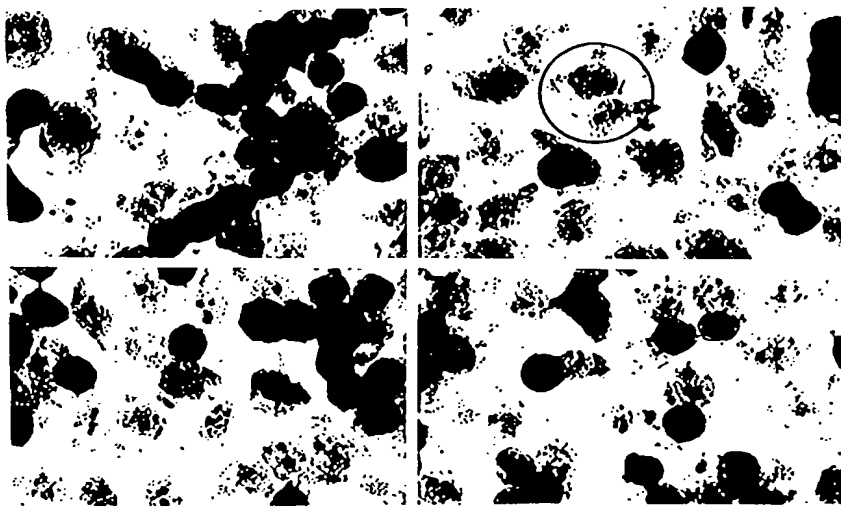


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/31, 15/62, C07K 14/22, 19/00, A61K 39/095	A3	(11) International Publication Number: WO 99/55875 (43) International Publication Date: 4 November 1999 (04.11.99)
(21) International Application Number: PCT/US99/09486 (22) International Filing Date: 29 April 1999 (29.04.99) (30) Priority Data: 60/083,405 29 April 1998 (29.04.98) US (71) Applicant (for all designated States except US): AMERICAN CYANAMID COMPANY [US/US]; Five Giralda Farms, Madison, NJ 07940 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): METCALF, Thomas, N., III [US/US]; 10 Caywood Lane, Fairport, NY 14450 (US). ZAGURSKY, Robert, J. [US/US]; 569 Fox Hunt Drive, Victor, NY 14564 (US). OOI, Peggy [US/US]; 494 Main Street Fishers, Mendon, NY 14506 (US). (74) Agents: GORDON, Alan, M.; American Home Products Corporation, Patent Law Dept. - 2B2, One Campus Drive, Parsippany, NJ 07054 (US) et al.		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 13 April 2000 (13.04.00)
(54) Title: VACCINES CONTAINING RECOMBINANT PILIN AGAINST <i>NEISSERIA GONORRHOEAE</i> OR <i>NEISSERIA MENINGITIDIS</i> 		
(57) Abstract <p>The <i>pilE</i> genes of each of <i>Neisseria gonorrhoeae</i> and <i>Neisseria meningitidis</i> are cloned and their corresponding recombinant pilin proteins are expressed. In addition, a chimeric <i>pilE</i> gene is constructed in which the region of the <i>pilE</i> gene of <i>Neisseria meningitidis</i> class I encoding the amino-terminal region of the pilin protein is replaced by the corresponding region of the <i>pilE</i> gene of <i>Neisseria gonorrhoeae</i>. The recombinant meningococcal chimeric class I pilin protein is expressed at higher levels than the pilin protein expressed by the full-length <i>pilE</i> gene of <i>Neisseria meningitidis</i>. Furthermore, a chimeric <i>pilE</i> gene is constructed in which the region of the <i>pilE</i> gene of <i>Neisseria meningitidis</i> class II encoding the carboxy-terminal region of the pilin protein is replaced by the corresponding region of the <i>pilE</i> gene of <i>Neisseria gonorrhoeae</i>. The recombinant pilin proteins are used in vaccines to protect against disease caused by <i>Neisseria gonorrhoeae</i> or <i>Neisseria meningitidis</i>.</p>		

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INTERNATIONAL SEARCH REPORT

Int. Application No.
PCT/US 99/09486

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/31 C12N15/62 C07K14/22 C07K19/00 A61K39/095

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUCHANAN, T.M. ET AL: "Development of a vaccine to prevent gonorrhea. BACTERIAL VACCINES.", SEMIN. INFECT. DIS., (1982) PP. 160-164. ISBN: 0-86577-020-0., U.S. PUBLIC HEALTH SERV. HOSP., SEATTLE, WA 98195, USA XP002118827	1,2,7-9, 15
Y	page 160, right-hand column -page 161, left-hand column page 162, right-hand column, line 26-33	7,8,13
X	US 4 443 431 A (BUCHANAN THOMAS M ET AL) 17 April 1984 (1984-04-17)	1,2,7-9, 15
Y	column 2, line 49-55 column 7, line 54 -column 8, line 8 table 2	7,8,13
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☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

10 February 2000

Date of mailing of the international search report

25.02.00

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INTERNATIONAL SEARCH REPORT

Intern. Application No.

PCT/US 99/09486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 00153 A (GARCON JOHNSON NATHALIE MARIE ;PRIEELS JEAN PAUL (BE); SMITHKLINE) 6 January 1994 (1994-01-06) the whole document page 5, line 9 claim 6	7,8
Y	GUPTA R K ET AL: "Adjuvants for human vaccines--current status, problems and future prospects" VACCINE, vol. 13, no. 14, 1 October 1995 (1995-10-01), page 1263-1276 XP004057427 ISSN: 0264-410X abstract page 1264, left-hand column, paragraph 2 page 1269, left-hand column, line 4-6 page 1271, left-hand column	7,8
Y	MARINARO ET AL.: "Interleukin-12 alters helper T-cell subsets and antibody profiles induced by the mucosal adjuvant cholera toxin" ANN N Y ACAD SCI, vol. 795, 31 October 1996 (1996-10-31), page 361-365 XP002117955 page 362, line 3-6 page 362, paragraph 3 -page 363, paragraph 1 page 364, paragraph 2	7,8
Y	WO 85 04654 A (SCRIPPS CLINIC RES) 24 October 1985 (1985-10-24) example 5	13
X	WO 94 08013 A (OREGON STATE) 14 April 1994 (1994-04-14)	1,3,4, 10,12,15
Y	page 3-6	5,6,11, 14, 28-30, 33-42
X	WO 93 11791 A (OREGON STATE) 24 June 1993 (1993-06-24)	1,3,4, 10,12,15
Y	page 5-7	5,6,11, 14, 28-30, 33-42

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INTERNATIONAL SEARCH REPORT

Instan Application No

PCT/US 99/09486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	POTTS & SAUNDERS: "Nucleotide sequence of the structural gene for class I pilin from <i>Neisseria meningitidis</i> : homologies with the <i>pilE</i> locus of <i>Neisseria gonorrhoeae</i> " MOLECULAR MICROBIOLOGY, vol. 2, no. 5, 1988, pages 647-653, XP002117231	16-27
Y	the whole document figure 3	6,11,14
X	VIRJI ET AL. : "Pilus-facilitated adherence of <i>Neisseria meningitidis</i> to human epithelial cells: modulation of adherence phenotype occurs concurrently with changes in primary amino acid sequence and the glycosylation status of pilin" MOLECULAR MICROBIOLOGY, vol. 10, no. 5, December 1993 (1993-12), pages 1013-1028, XP000867896	16-27
Y	abstract figure 6	6,11,14
X	AHO ET AL.: "Characterization of a class II pilin expression locus from <i>Neisseria meningitidis</i> : evidence for increased diversity among pilin genes in pathogenic <i>Neisseria</i> species" INFECTION & IMMUNITY, vol. 65, no. 7, July 1997 (1997-07), pages 2613-2620, XP002129888	31,32
Y	cited in the application abstract page 2617 -page 2618 figures 2,3 table 1	5,28-30, 33-42
A	EP 0 049 945 A (BACTEX INC) 21 April 1982 (1982-04-21) example 7 claims 3,4,7	1-5,9, 10,12, 13,15
A	PERRY ET AL.: "Structural analysis of the <i>pilE</i> region of <i>Neisseria gonorrhoeae</i> P9" GENE, vol. 60, 1987, pages 85-92, XP002117230 abstract figure 3	1,2,7-9, 13,15
	-/--	

INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/US 99/09486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SEIFERT: "Questions about gonococcal pilus phase- and antigenic variation" MOLECULAR MICROBIOLOGY, vol. 21, no. 3, 1996, pages 433-440, XP002117233 the whole document page 437, right-hand column	1,2
A	NASSIF ET AL: "Type-4 pili and meningococcal adhesiveness" GENE, vol. 192, no. 1, 11 June 1997 (1997-06-11), page 149-153 XP004115699 ISSN: 0378-1119 abstract page 151-152, paragraphs 4,6	1,3-5
A	MAKRIDES S C: "Strategies for Achieving High-Level Expression of Genes in Escherichia coli" MICROBIOLOGICAL REVIEWS, US, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, vol. 60, no. 3, 1 September 1996 (1996-09-01), pages 512-538, XP002095235 ISSN: 0146-0749 page 512 -page 518, left-hand column page 524	6,18-28, 33-42
A	CARRICK C S ET AL: "The normally silent sigma promoters upstream of the pili genes of both Neisseria gonorrhoeae and Neisseria meningitidis are functional when transferred to Pseudomonas aeruginosa" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, GB, ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 198, no. 1-2, 1 October 1997 (1997-10-01), pages 89-97, XP004116043 ISSN: 0378-1119 abstract figure 1	6
A	WO 92 13871 A (UNIV WASHINGTON) 20 August 1992 (1992-08-20) the whole document	1
A	WO 96 31618 A (UNIV NORTH CAROLINA) 10 October 1996 (1996-10-10) the whole document	1
	-/--	

INTERNATIONAL SEARCH REPORT

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PCT/US 99/09486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	<p>EP 0 474 313 A (CIGB) 11 March 1992 (1992-03-11) the whole document</p>	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/09486

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 9-14, 29 and 30 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,15 (all partially); 2,7,8,9,13 (all completely)

A vaccine composition comprising isolated and purified recombinantly-expressed pilin protein from the species *Neisseria gonorrhoeae*, further comprising an adjuvant, diluent or carrier, the adjuvant being selected from the group consisting of aluminium hydroxide, aluminium phosphate, Stimulon QS-21, 3-O-deacylated monophosphoryl lipid A, Il-12, and wild-type or mutant cholera toxin, and methods of immunisation and preparation.

2. Claims: 1,3,10,12,15 (all partially); 4 (completely)

A vaccine composition comprising isolated and purified recombinantly-expressed class I pilin protein from the species *Neisseria meningitidis*, and methods of immunisation and preparation.

3. Claims: 1,3,10,12,15 (all partially); 5 (completely)

A vaccine composition comprising isolated and purified recombinantly-expressed class II pilin protein from the species *Neisseria meningitidis*, and methods of immunisation and preparation.

4. Claims: 1,15 (all partially); 6,11,14,
16-42 (all completely)

- A vaccine composition comprising isolated and purified recombinantly-expressed chimeric pilin protein of *Neisseria gonorrhoeae* and class I *Neisseria meningitidis* having the amino acid sequence 1-167 (or 8-167 after maturation) of SEQ ID NO:2 or a biologically equivalent amino acid sequence, and methods of immunisation and preparation.

Isolated and purified encoding nucleic acids and variants (SEQ ID NO:1), plasmids, host cells, methods of production, and the isolated and purified chimeric pilin.

- A vaccine composition comprising isolated and purified recombinantly-expressed chimeric pilin protein of *Neisseria gonorrhoeae* and class II *Neisseria meningitidis* having the amino acid sequence 1-170 (or 8-170 after maturation) of SEQ ID NO:4 or a biologically equivalent amino acid sequence, and methods of immunisation and preparation.

Isolated and purified encoding nucleic acids and variants (SEQ ID NO:3), plasmids, host cells, methods of production, and the isolated and purified chimeric pilin.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte Application No

PCT/US 99/09486

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 4443431	A	17-04-1984	NONE	
WO 9400153	A	06-01-1994	AP 408 A	27-09-1995
			AT 156710 T	15-08-1997
			AU 1785597 A	19-06-1997
			AU 661404 B	20-07-1995
			AU 4326393 A	24-01-1994
			AU 676166 B	06-03-1997
			AU 4326493 A	24-01-1994
			CA 2138996 A	06-01-1994
			CA 2138997 A	06-01-1994
			CN 1086142 A	04-05-1994
			CN 1092812 A	28-09-1994
			CZ 9403296 A	16-08-1995
			DE 69313134 D	18-09-1997
			DE 69313134 T	26-02-1998
			DK 671948 T	01-09-1997
			WO 9400575 A	06-01-1994
			EP 0671948 A	20-09-1995
			EP 0649470 A	26-04-1995
			EP 0761231 A	12-03-1997
			ES 2108278 T	16-12-1997
			FI 946064 A	22-02-1995
			GR 3025184 T	27-02-1998
			HU 71208 A	28-11-1995
			IL 106109 A	18-02-1997
			JP 7508512 T	21-09-1995
			JP 7508648 T	28-09-1995
			MX 9303771 A	31-05-1994
			MX 9303773 A	31-05-1994
			NO 945003 A	23-12-1994
			NZ 253137 A	27-08-1996
			NZ 253138 A	26-10-1995
			PL 170980 B	28-02-1997
			SG 49909 A	15-06-1998
			SI 9300335 A	31-12-1993
			SK 159294 A	09-08-1995
			US 5750110 A	12-05-1998
WO 8504654	A	24-10-1985	AU 582358 B	23-03-1989
			AU 4159085 A	01-11-1985
			DK 565285 A	05-12-1985
			EP 0177583 A	16-04-1986
			FI 854839 A, B,	05-12-1985
			IL 74829 A	28-02-1989
			JP 61501777 T	21-08-1986
			NO 854903 A	04-02-1986
			NZ 211715 A	27-01-1989
			ZA 8502629 A	27-11-1985
WO 9408013	A	14-04-1994	AU 5403594 A	26-04-1994
WO 9311791	A	24-06-1993	AU 3416693 A	19-07-1993
EP 0049945	A	21-04-1982	EP 0048422 A	31-03-1982
			EP 0048881 A	07-04-1982
			JP 1932813 C	26-05-1995
			JP 6062432 B	17-08-1994

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter Application No

PCT/US 99/09486

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0049945 A		JP 57081415 A US 4769240 A	21-05-1982 06-09-1988
WO 9213871 A	20-08-1992	AU 1411492 A US 5834591 A	07-09-1992 10-11-1998
WO 9631618 A	10-10-1996	AU 5537096 A CA 2217522 A EP 0830456 A JP 11503322 T	23-10-1996 10-10-1996 25-03-1998 26-03-1999
EP 0474313 A	11-03-1992	CU 22302 B AT 152175 T AU 657487 B AU 8368391 A CA 2050749 A DE 69125769 D DE 69125769 T ES 2103295 T FI 914129 A GR 3024127 T JP 6169779 A US 5286484 A	02-12-1994 15-05-1997 16-03-1995 12-03-1992 08-03-1992 28-05-1997 27-11-1997 16-09-1997 08-03-1992 31-10-1997 21-06-1994 15-02-1994

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